Pergamon

S0031-9422(96)00244-0

Phytochemistry, Vol. 42, No. 6, pp. 1583–1586, 1996

0031-9422/96 $15.00 + 0.00

BIOTRANSFORMATION OF CEDROL AND RELATED COMPOUNDS BY *MUCOR PLUMBEUS*

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(*Received in revised form* 10 *January* 1996)

Key Word Index—*Mucor plumbeus*; microbiological hydroxylation; cedrol; sesquiterpenoid.

Abstract—The hydroxylation of cedrol, 8-epicedrol, 9α-hydroxycedrane and 8α,9α-dihydroxycedrane by *Mucor plumbeus* has been shown to proceed efficiently, but not stereospecifically, at the C-3 position. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The ability of microorganisms to hydroxylate chemically inaccessible centres is potentially a powerful synthetic tool which has been realized in the steroid area, but to a far lesser extent elsewhere. One of the reasons for this is the lack of predictive models that relate plausible sites of hydroxylation to potential substrate structures. The steroids are relatively flat molecules, and models derived from this area suggest triangular relationships between binding and hydroxylated centres [1–3]. In this context the biotransformation of the variety of bridged ring polycyclic sesquiterpenoids [3] provides a useful way of eventually mapping the three-dimensional topology of microbial systems. The cedrane carbon skeleton possesses a well-defined rigid bridged system, which is one that is suitable for this purpose. Because of the experimental difficulties that are associated with the isolation and application on a preparative scale of all but a limited number of microbial hydroxylases, synthetically useful predictive models currently need to be constructed in terms of the hydroxylating ability of intact organisms. The synthetic utility of an organism may well be the summation of the activity of several discrete enzyme systems. The fungus *Mucor plumbeus* [1–3] has proved to be a useful organism for microbiological hydroxylation, although its capabilities are not yet fully mapped. We have, therefore, explored the microbiological hydroxylation of cedrol (**1**), 8-epicedrol (**12**), 9α-hydroxycedrane (**19**) and 8α-9α-dihydroxycedrane (**23**) by *Mucor plumbeus*. In these compounds the position and stereochemistry of a plausible directing hydroxyl group has been varied.

The microbiological hydroxylation of (**1**) by *Aspergillus niger* [4], *Beauveria sulfurescens* [5], *Cephalosporium aphidicola* [6, 7] and *Glomerella cingulata* [8] has been shown to take place predominantly at C-3. In other studies [9] with *Rhizopus stolonifer*, *Streptomyces bikiniensis*, *Verticillum tenerum*, *Streptoverticillium reticuli* and *Corynespora cassiocola*, hydroxylation was less regiospecific, taking place at C-2, C-3, C-4, C-9, C-10 and C-12 with attack at C-2 and C-12 predominating.

RESULTS AND DISCUSSION

Cedrol (**1**) was available commercially whilst the other substrates were prepared from α-cedrene by literature methods [10] involving epoxydation and reduction for **12**, hydroboronation for **19** and osmylation for **23**. The substrates were added to one-day-old shake cultures of *M. plumbeus*. The metabolites were isolated after a further six days and the results are presented in Table 1. In a number of cases the mixed fractions were separated after acetylation. The known [5–7] compounds, **2**, **3**, **4**, **13**, **14**, **15**, **19** and **20**, were identified by their ^{1}H NMR spectra.

The structure of 9α,13-dihydroxycedrane (**22**) followed from the ^{1}H NMR spectrum, which contained a pair of AB doublets (J = 11 Hz) at δ_H 3.30 and 3.49, characteristic of a primary alcohol. One of the singlet methyl group resonances of the starting material was missing. The ^{13}C NMR spectrum contained a CH_2-O signal at δ_C 69.82. The stereochemistry of this primary alcohol was established by a NOE experiment. Irradiation of the singlet methyl group signal at δ_H 1.20 produced an enhancement of 11.2% at H-9β and 4% at δ_H 3.30. Hence, the primary school was located at C-13 rather than at C-14. The C-3α and C-3β epimeric alcohols, **2** and **3**, were clearly distinguished since the H-3β ^{1}H NMR signal appears as a triplet (J = 9.5 Hz) of doublets (J = 5.1 Hz) at δ_H 3.65, whilst the H-3α signal appears as a double doublet (J = 4.1 and 8.1 Hz)

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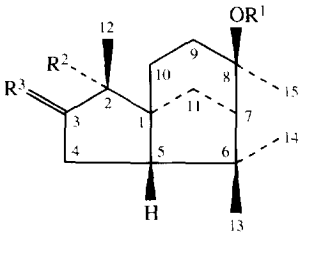
Table 1. Hydroxylation of cedrol derivatives by *Mucor plumbeus*

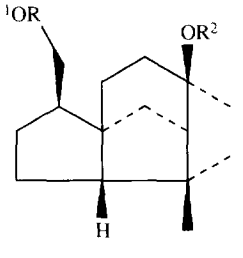
Substrate	Product	Yield (%)
Cedrol (**1**)	3α,8β-Dihydroxycedrane (**2**)	67
	3β,8β-Dihydroxycedrane (**3**) (1:1 mixture)	
	2α,8β-Dihydroxcedrane (**4**)	3
	8β,12-Dihydroxycedrane (**6**)	2
	3β,8β,10-Trihydroxycedrane (?)(**9**)	1.5
8-Epicedrol (12)		
	3α,8α-Dihydroxycedrane (**13**)	20
	3β,8α-Dihydroxycedrane (**14**)	15
	3α,Hydroxycedr-8-ene (**15**)	10
	15α-Dihydroxycedr-8-ene (**16**)	6
9α-Hydroxycedrane (**19**)		
	3α,9α-Dihydroxycedrane (**20**)	17
	3β,9α-Dihydroxycedrane (**21**)	12
	9α,13-Dihydroxycedrane (**22**)	6
8α-9α-Dihydroxycedrane (**23**)		
	3α,8α,9α-Trihydroxycedrane (**24**)	63

at δ_H 4.29. The ^{1}H NMR spectrum of 3α,15-dihydroxycedr-8-ene (**16**) was very similar to that of the known [6] 3β,15-dihydroxycedr-8-ene except that the H-3α resonance of the latter was replaced by the typical H-3β signal at δ_H 3.71. Acetylation gave a separable mixture of a 15-monoacetate (**17**) and a 3α,15-diacetate (**18**). The structure of 3α,8α,9α-trihydroxycedrane (**24**) also followed from the ^{1}H NMR spectrum, which contained the characteristic H-3β signal (δ_H 3.58, triplet, J = 10.1 Hz, of doublets, J = 5.2 Hz). Irradiation of the methyl group doublet, δ_H 0.98 (H-12), produced a NOE enhancement of 5.7% at δ 3.58. Irradiation of the H-15 methyl group singlet (δ_H 1.37) produced a NOE enhancement of 3.5% at δ_H 3.69 (H-9) and 2.4% at the methyl group singlet, δ_H 1.14 (H-13). Irradiation of this signal gave a NOE enhancement of 10.3% at δ_H 3.69 (H-9), 4.0% at δ_H 1.37 (H-15) and 1.3% at δ_H 1.03 (H-14).

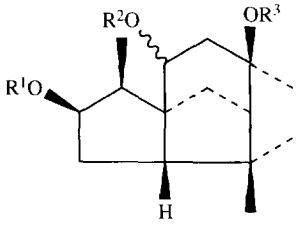
An interesting feature of these transformations is their efficiency and the tendency of *M. plumbeus* to hydroxylate at C-3 irrespective of the stereochemistry of the hydroxyl group at C-8. This feature was noted in the hydroxylations of this series by *C. aphidicola* [7]. It suggests that substrate/enzyme interactions, involving polar regions rather than polar centres, may exert a directing effect on the regiochemistry of hydroxylation. However, unlike many microbiological hydroxylations, hydroxylation at C-3 was not stereospecific.



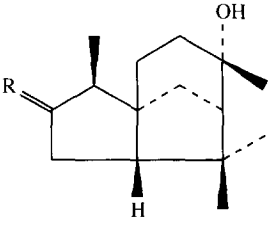
1 $R^1 = R^2 = H$, $R^3 = H_2$
2 $R^1 = R^2 = H$, R^3 = α-OH, β-H
3 $R^1 = R^2 = H$, R^3 = α-H, β-OH
4 $R^1 = H$, $R^2 = OH$, $R^3 = H_2$
5 $R^1 = Ac$, $R^2 = OAc$, $R^3 = H_2$



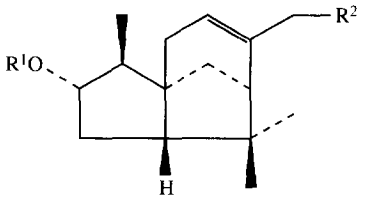
6 $R^1 = R^2 = H$
7 $R^1 = Ac$, $R^2 = H$
8 $R^1 = R^2 = Ac$

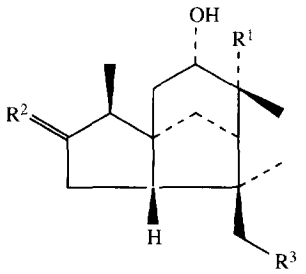


9 $R^1 = R^2 = R^3 = H$
10 $R^1 = R^2 = Ac$, $R^3 = H$
11 $R^1 = R^2 = R^3 = Ac$



12 $R = H_2$
13 R = α-OH, β-H
14 R = α-H, β-OH





15 $R^1 = R^2 = H$
16 $R^1 = H$, $R^2 = OH$
17 $R^1 = H$, $R^2 = OAc$
18 $R^1 = Ac$, $R^2 = OAc$

19 $R^1 = R^3 = H$, $R^2 = H_2$
20 $R^1 = R^3 = H$, $R^2 = \alpha$-OH, β-H
21 $R^1 = R^3 = H$, $R^2 = \alpha$-H, β-OH
22 $R^1 = H$, $R^2 = H_2$, $R^3 = OH$
23 $R^1 = OH$, $R^2 = H_2$, $R^3 = H$
24 $R^1 = OH$, $R^2 = \alpha$-OH, β-H, $R^3 = H$

EXPERIMENTAL

General experimental details have been described previously [11]. *M. plumbeus* was grown on shake culture on the following medium (100 ml in 250 ml conical flasks) (l^{-1}) glucose (80 g), NH_4NO_3 (0.48 g), KH_2PO_4 (5 g), $MgSO_4$ (1 g) and trace elements soln (2 ml). The trace elements soln contained (per 100 ml) $Co(NO_3)_2$ (0.01 g), $FeSO_4$ (0.1 g), $CuSO_4$ (0.015 g), $ZnSO_4$ (0.16 g), $MnSO_4$ (0.01 g) and NH_4 molybdate (0.01 g). The substrate in EtOH (20 ml) was evenly distributed between 45 flasks after 1 days' growth. After a further 6 days, the fermentation was harvested. The mycelium was filtered and the culture filtrate was extracted with EtOAc. THe extract was dried over Na_2SO_4 the solvent was evapd and the residue was chromatographed on a silica gel column in an EtOAc–petrol gradient.

Incubation of cedrol (**1**). Cedrol (900 mg) gave, after chromatography, a mixt. (1:1 by ^{1}H NMR) of 3α- and 3β,8β-dihydroxycedrane (650 mg). A portion (30 mg) was subjected to further column chromatography on silica gel in CH_2Cl_2–EtOAc (5:1) to yield 3α,8β-dihydroxycedrane (**2**) (5 mg) and 3β,8β-dihydroxycedrane (**3**) (5 mg) identified by their ^{1}H NMR spectra [6]. Acetylation of a further mixt. with Ac_2O–pyridine at room temp. and repeated chromatography in EtOAc–petrol gave 12-acetoxy-8β-hydroxycedrane (**7**) (14 mg) [6] and 2α,8β-dihydroxycedrane (**4**) (20 mg) [6] identified by their ^{1}H NMR spectra. Acetylation at 70° gave 2α,8β-diacetoxycedrane (**5**) (8 mg) and 8β,12-diacetoxycedrane (**8**) (6 mg). Acetylation of a further mixt., eluted with EtOAc–petrol (7:2) gave compounds which were tentatively identified as 3β,10ξ-diacetoxy-8β-hydroxycedrane (**10**) (15 mg) and 3β,8β,10ξ-triacetoxycedrane (**11**) (4 mg).

Incubation of 8-epicedrol (**12**). 8-Epicedrol (600 mg) gave 3α-hydroxycedr-8-ene (**15**) (65 mg) [6], 3α,8α-dihydroxycedrane (**13**) (130 mg) [7] and 3β,8α-dihydroxycedrane (**14**) (100 mg) [7] identified by their ^{1}H NMR spectra. Further elution gave 3α,15-dihydroxycedr-8-ene (**16**) (35 mg). Acetylation with Ac_2O–pyridine at room temp. gave the 15-monoacetate (**17**) (12 mg) and the 3α,15-diacetate (**18**) (4 mg).

Incubation of 9α-hydroxycedrane (**19**). 9α-Hydroxycedrane (**19**) (540 mg) gave 3α,9α-dihydroxycedrane (**20**) (100 mg) [7], 3β,9α-dihydroxycedrane (**21**) (70 mg) [7] and 9α,13-dihydroxycedrane (**22**) (35 mg).

Incubation of 8α,9α-dihydroxycedrane (**23**). 8α,9α-Dihydroxycedrane (430 mg) gave 3α,8α,9α-trihydroxycedrane (**24**) (290 mg). ^{1}H NMR spectra of the crude material indicated the presence of a small amount of the 3β-isomer.

Characterization of new compounds. 2α,8β-Diacetoxycedrane (**5**), oil, ^{1}H NMR ($CDCl_3$): δ 1.01, 1.16, 1.37, 1.62 (each 3H, *s*), 1.96, 1.98 (each 3H, s, OAc). MS *m/z* (rel. int.): 262 $[M-60]^+$ (6), 220 (5), 202 (100), 187 (35), 173 (18), 159 (74), 145 (32). 8β,12-Diacetoxycedrane (**8**), oil, ^{1}H NMR ($CDCl_3$): δ 1.00, 1.19, 1.55 (each 3H, *s*), 1.96, 2.05 (each 3H, *s*, OAc), 3.96, 4.05 (1H, each *dd*, $J = 7.2$, 11 Hz). MS *m/z* (rel. int.): 262 $[M-60]^+$ (33), 247 (1), 233 (4), 202 (68), 187 (29), 173 (30), 159 (100), 145 (39). 3β,10ξ-Diacetoxy-8β-hydroxycedrane (**10**), oil, ^{1}H NMR ($CDCl_3$): δ 0.86 (3H, *d*, $J = 7.4$ Hz), 1.04, 1.32, 1.37 (each 3H, *s*), 2.07, 2.08 (each 3H, *s*, OAc), 4.82 (1H, *dd*, $J = 6.3$, 10 Hz), 5.26 ((1H, *ddd*, $J = 1.8$, 4.3, 4.8 Hz). MS *m/z* (rel. int.): 296 $[M-42]^+$ (1), 278 (3), 263 (5), 260 (5), 236 (12), 218 (45), 203 (25), 200 (40), 185 (34), 175 (45), 145 (100).

3β,8β,10-Triacetoxycedrane (**11**) Oil, ^{1}H NMR: δ 0.85 (3H, *d*, $J = 7.5$ Hz), 1.03, 1.22, 1.58 (each 3H, *s*), 1.96, 2.06, 2.08 (each 3H, *s*, OAc), 4.85 (1H, *dd*, $J = 6.2$, 10 Hz), 5.26 (1H, *ddd*, $J = 2.0$, 4.2, 4.6 Hz). MS *m/z* (rel. int.): 320 $[M-60]^+$ (2), 305 (1), 278 (2), 263 (4), 260 (23), 218 (16), 200 (94), 185 (61), 157 (79), 131 (100). Both **10** and **11** are tentative structures.

3α,15-Dihydroxycedr-8-ene (**16**), mp 139–142°. IR ν_{max} cm^{-1} 3242. ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, *d*, *J* = 6.9 Hz), 1.01, 1.04 (each 3H, *s*), 3.71 (1H, *t* of *d*, *J* = 10, 5.4 Hz), 3.99, 4.05 (each 1H, *d*, *J* = 13.9 Hz), 5.49 (1H, *br.s*). MS *m/z* (rel. int.): 236 (30), 218 (25), 200 (10), 187 (30), 163 (60), 131 (65), 118 (100). 15-Acetoxy-3α-hydroxycedr-8-ene (**17**), oil. ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, *d*, *J* = 7 Hz), 1.01, 1.06 (each 3H, *s*), 2.08 (3H, *s*, OAc), 3.71 (1H, *t* of *d*, *J* = 10, 5.4 Hz), 4.43, 4.48 (each 1H, *d*, *J* = 13 Hz), 5.54 (1H, *br.s*). MS *m/z* (rel. int.): 278 (1), 260 (1), 236 (8), 200 (18), 185 (9), 157 (26). 3α,15-Diacetoxycedr-8-ene (**18**), oil, ^{1}H NMR ($CDCl_3$); δ 0.91 (3H, *d*, *J* = 7.1 Hz) 1.00, 1.05 (each 3H, *s*), 2.06, 2.09 (each 3H, *s*, OAc), 4.43, 4.49 (each 1H, *d*, *J* = 13.5 Hz), 4.74 (1H, *t* of *d*, *J* = 9.2, 5.6 Hz), 5.55 (1H, *br s*). MS *m/z* (rel. int.): 320 (1), 278 (8), 260 (6), 245 (1), 200 (52), 185 (20). 9α-Dihydroxycedrane (**22**), mp 178–181°. IR ν_{max} cm^{-1}: 3585 (br). ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, *d*, *J* = 7.1 Hz), 1.15 (3H, *d*, *J* = 7.2 Hz), 1.20 (3H, *s*), 3.30, 3.49 (each 1H, *d*, *J* = 11 Hz), 3.76 (1H, *t* of *d*, *J* = 10, 6 Hz); ^{13}C NMR: δ_C 15.40 (C-12), 17.63 (C-15), 22.49 (C-14), 24.59 (C-4), 37.46 (C-3), 41.89 (C-2), 43.65 (C-11), 45.89 (C-8), 46.80 (C-10), 47.95 (C-6), 49.20 (C-7), 54.35 (C-1), 58.92 (C-5), 69.82 (C-13), 72.79 (C-9); MS 238 (15), 220 (15), 205 (8), 187 (8), 167 (40), 123 (100). 3α,8α,9α-Trihydroxycedrane (**24**), mp 239–242°. IR ν_{max} cm^{-1} 3584 (sh), 3236 (br). ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, *d*, *J* = 7.1 Hz), 1.03, 1.14, 1.37 (each 3H, *s*), 3.58 (1*H*, *t* of *d*, *J* = 10.1, 5.2 Hz), 3.69 (1H, *dd*, *J* = 10.5, 6.4 Hz). ^{13}C NMR: δ_C 12.06 (C-12), 26.28 (C-13), 28.03 (C-14), 29.52 (C-15), 34.99 (C-4), 39.93 (C-10), 40.54 (C-11), 40.54 (C-6), 49.49 (C-2), 49.78 (C-1), 53.40 (C-7), 60.43 (C-5), 70.94 (C-9), 73.83 (C-8), 80.71 (C-3). MS *m/z* (rel. int.): 254 (10), 236 (25), 218 (25), 191 (30), 166 (60), 149 (50).

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